



Expression of Proteins in *E. coli*

Expression of a recombinant protein can be approached in general by constructing a plasmid that encodes the desired protein, introducing the plasmid into the required host cell, growing the host cells and inducing protein expression, and then lysing the cells, purifying the protein, and performing SDS-PAGE analysis to verify the presence of the protein (Figure 1).

The protocols and recommendations given in the Plasmid DNA chapter for the handling and transformation of *E. coli* are also valid for the production of recombinant proteins. With careful choice of host strains, vectors, and growth conditions, most recombinant proteins can be cloned and expressed at high levels in *E. coli*.

Optimal growth and expression conditions for the protein of interest should be established with small-scale cultures before large-scale protein purification is attempted.

Generation of Recombinant Proteins

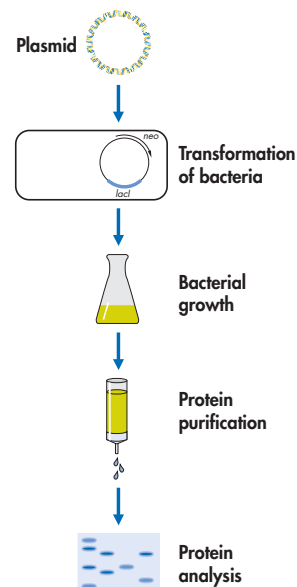


Figure 1. Overview of the steps involved in expression and analysis of recombinant proteins.

Basic principles

This section discusses critical factors to be considered when expressing foreign proteins in *E. coli*.

Culture media

The media of choice for the growth of *E. coli* cells containing an expression plasmid are LB medium and its modifications, 2x YT, or Super Broth, each containing the relevant selective antibiotic(s). Initially it is advisable to try expression in all three media in parallel, and to do a time course analysis to monitor growth and expression after induction. Striking differences between the level of expression in different media and at different times are often observed.

Maintenance of the expression plasmid

Poor plasmid maintenance in the cells can lead to low expression levels. Ampicillin is an unstable antibiotic and is rapidly depleted in growing cultures due in part to the β -lactamase secreted by resistant bacterial cells. It is important to check plasmid levels by plating cells from the expression culture on plates with and without ampicillin. If the stability of the expression construct is a problem, the cultures should be grown in the presence of 200 μ g/ml ampicillin, and the level should be maintained by supplementing ampicillin during long growth periods. Alternatively, the cultures may be grown in the presence of carbenicillin, a more stable β -lactam, at 50 μ g/ml (see "Antibiotics", page 5).



Small-scale expression cultures

Small-scale expression and purification experiments are highly recommended and should be performed before proceeding with a large-scale preparation. In many cases aliquots of the cells can be lysed in a small volume of sample buffer and analyzed directly by SDS-PAGE. The use of small expression cultures provides a rapid way to judge the effects of varied growth conditions on expression levels and solubility of recombinant proteins. Expression levels vary between different colonies of freshly transformed cells, and small-scale preparations permit the selection of clones displaying optimal expression rates.

Induction of protein expression

The method used for induction of protein expression is dependent on the plasmid vector and *E. coli* strain used. Protein expression can be induced by a raising of the incubation temperature or by the addition of an inducing chemical such as isopropyl- β -D-thiogalactoside (IPTG) to the culture medium. Details of induction methods and the plasmids they relate to can be found in standard molecular biology texts (1,2).

Time-course analysis of protein expression

To optimize the expression of a given protein construct, a time-course analysis by SDS-PAGE (Protocol 5, page 75) of the level of protein expression is recommended. Intracellular protein content is often a balance between the amount of soluble protein in the cells, the formation of inclusion bodies, and protein degradation. By checking the protein present at various times after induction, the optimal induction period can be established (Figure 2).

Time-Course Analysis of Protein Expression

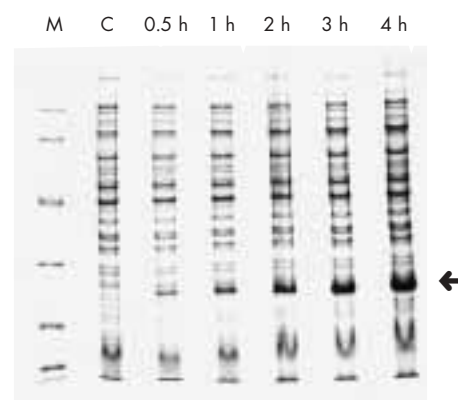


Figure 2. Time course analysis of the expression of dihydrofolate reductase (arrowed). Aliquots were removed at the times indicated and analysed by SDS-PAGE. **C:** uninduced control. **M:** markers.

Colony blots

We recommend the colony-blot procedure (Figure 3; Protocol 1, page 72) to identify clones expressing a protein and to distinguish semi-quantitatively between expression rates. This can be an advantage for selecting clones after transformation, since freshly transformed colonies may differ significantly in their expression rates. Using this method, colonies subsequently found to be expressing proteins at rates as low as 0.1 to 0.5 mg/liter are easily distinguished from colonies that do not express protein.

Note: If using the QIAexpress® Expression System, the small size of the His tag means that small peptides (<30 amino acids) expressed from QIAexpress vectors without an insert are degraded within the cells, and will not yield a false positive signal in the detection procedure. Other commonly used vectors that encode larger affinity tags may lead to expression of a small, but stable and detectable translation product even without an insert. This will lead to false positive signals from colonies that harbor the expression vector without insert, which may be indistinguishable from the signals from colonies expressing the desired protein.

Colony-Blot Procedure

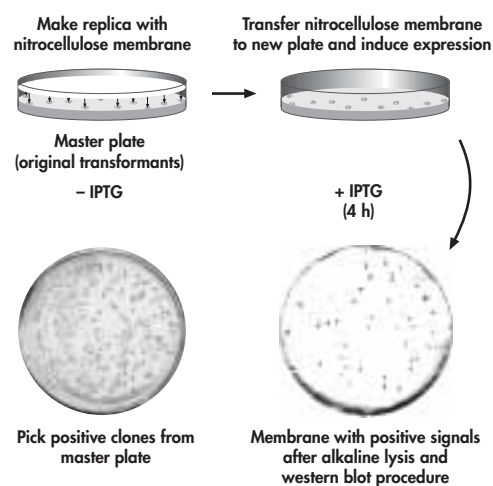


Figure 3. Detection of positive expression clones by colony blotting.